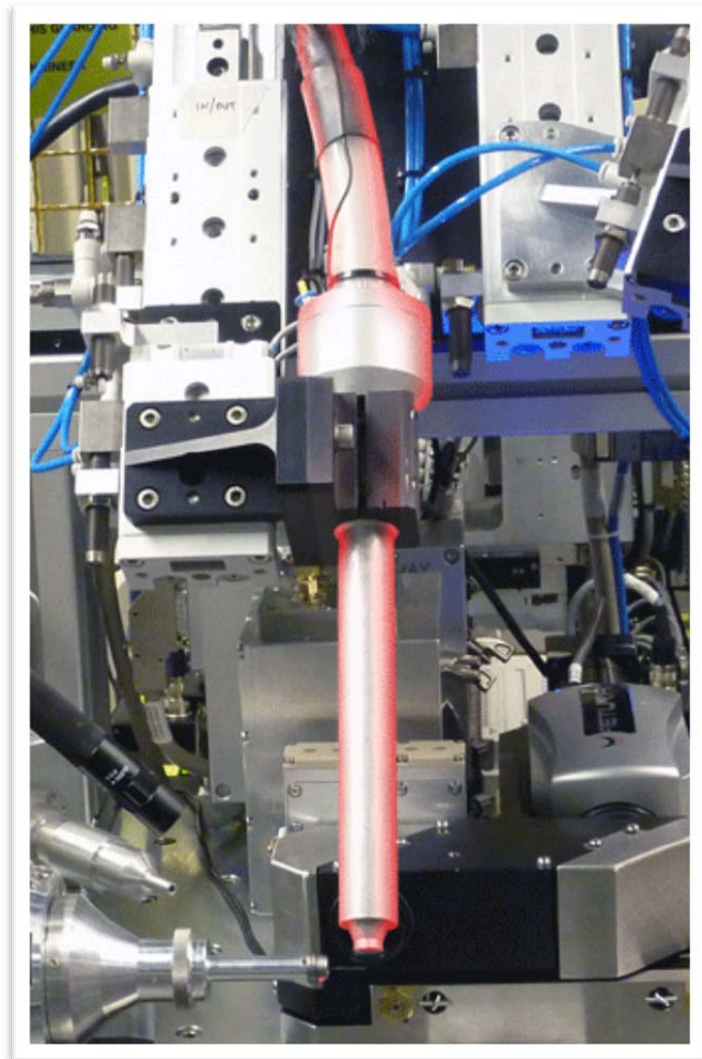


HC1 USER MANUAL



February 2015



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SECTION I: EXPERIMENTAL CONSIDERATIONS WHEN USING THE HC1

Introduction

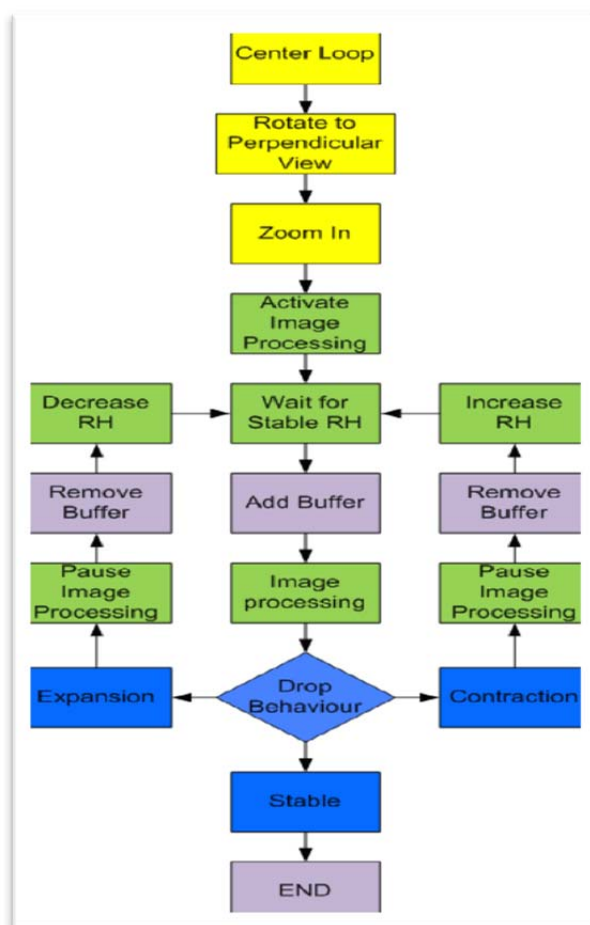
A dehydration experiment requires some time as samples are variable, there are numerous options and the analysis can be difficult if the effect of radiation damage and cryo-cooling are not properly taken into account. The focus of the experiment should be on trying to understand the effect on global diffracting parameters rather than solely focused on resolution. The measurements and behaviour observed may lead to follow-up experiments that may ultimately lead to improved diffraction. In this manual, we present some of the basic aspects of undertaking a hydration/dehydration experiment with the HC1 at a beamline at Diamond Light Source and give pointers to how to later undertake an offline experiment with the HC1 and/or *in-situ* at the home laboratory.

Several global stages need to be followed in order to be able to undertake any experiment. They will be described here in practical terms trying to give an insight into the variations that can be tried in order to undertake a successful experiment. There will also be a description of how the beamline and laboratory software are used. The key stages are described below.

Determining the RH_i and the RH_{target}

RH_i or initial Relative Humidity is the relative humidity at which a crystal is stable in the HC1 airstream. The RH_i is measured by empirically determining the behaviour of a droplet of mother liquor in the HC1 airstream. If the droplet expands the humidity is too high, if it shrinks it is too low and it is correct when the droplet remains stable.

The RH_i value will depend on the chemical composition of the solution. Attempts have been made to tabulate the most common precipitants used in crystallography¹. Using the theoretical and empirical data there is a web server available² that can be used to find the starting point. It is always advisable to measure check the actual RH_i before each experiment on the day as samples might have changed since last time or the device performance may vary slightly on the day.



¹ Russi, S., D. H. Juers, et al. (2011). "Inducing phase changes in crystals of macromolecules: Status and perspectives for controlled crystal dehydration." *J Struct Biol*.

² <http://www.embl.fr/CrystalDehydrationCollaboration/RH.html>

What solution to use for RH_i and RH_{target} ?

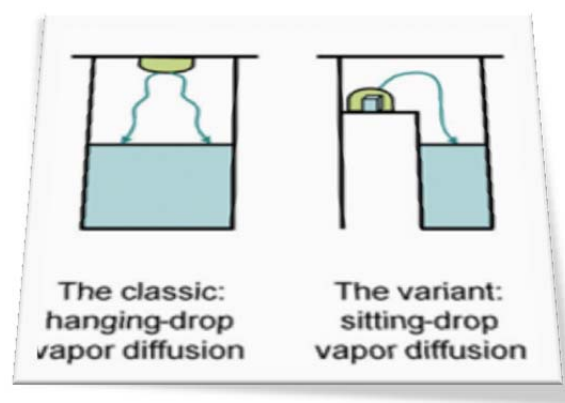
Crystals are grown, in most cases, in vapour equilibrium with a solution buffer and precipitant. Thus, this is a good solution to use for determining the RH_i . Using the actual liquid surrounding the crystals for RH_i determination is in most cases impractical and unnecessary. If possible, use it to fine-tune the RH_i value at the end of the process.

Solutions containing highly volatile compounds (alcohols/buffers) may be problematic if these compounds are essential for maintaining the lattice stability of your samples. This is because the HC1 can only use controlled water vapour to keep samples stable.

If crystals grow in a narrow range of precipitant concentrations, use the highest concentration well to determine the RH_i . If crystals grow in a broad range of buffer concentrations, it may necessary to sample a couple of points at the extremes and estimate the best value for each solution. It is always better to slightly dehydrate the samples than to hydrate them, since in hydrating the crystal you risk dissolving it.

The HC1 can only operate at room temperature so if the crystals are grown at low temperatures (4 °C usually) samples may behave oddly. Non-linear behaviour may be seen, as vapour pressure will change as the buffer warms up. If crystals require low temperature for their lattice to remain stable, they may dissolve during the process and thus not be suitable for the experiments.

It is worth noting that there is a link between cryo-protecting capacity and dehydration. Solutions that are not cryoprotectants tend to require higher humidity than those that are cryo-protected. In addition, if a sample is dehydrated below 94-95 % RH there is normally no apparent hexagonal ice formation upon cryo-cooling (as judged by X-ray diffraction) and this can be used as a cryo-cooling method³. Furthermore, when transferring crystal to cryo-protecting solution during standard cryo work samples frequently undergo a dehydration process that can alter the diffraction properties of the crystal and often result in damage often requiring testing numerous protocols prior finding the ideal cryo-cooling process. A known "good cryo" is a good buffer solution to measure equilibrium humidity to use as the first RH_{target} . Dehydrating samples to this RH will induce dehydration in the samples that can be expected to be possible and compatible with viable data. Also, depending on how different the value is from the starting point, it gives an indication of how fast the dehydration may be possible as samples that are stable in very dehydrating cryo solutions can probably be dehydrated relatively quickly (2-5 %/minute).



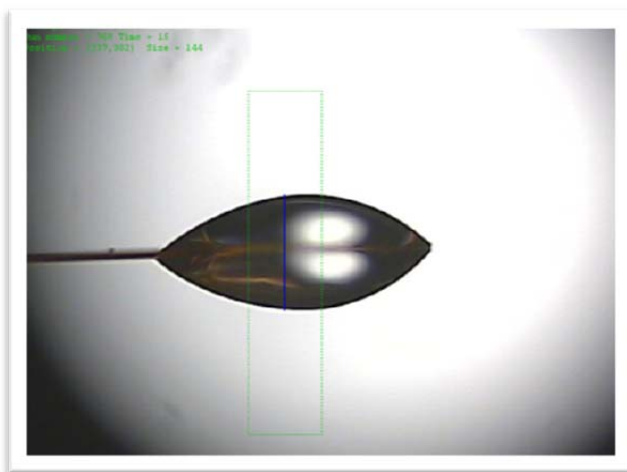
³ Wheeler, M. J., S. Russi, *et al.* (2012). "Measurement of the equilibrium relative humidity for common precipitant concentrations: facilitating controlled dehydration experiments." *Acta Crystallogr Sect F Struct Biol Cryst Commun* **68**(1): 111-114.

Measuring the RH_i

Place a large Kapton® loop (300-900 µm) on the goniometer stage. Using the beamline software, centre the loop and rotate it so that it appears perpendicular to the field of view. Once there, dispense liquid onto the loop such that it only creates an ellipsoid droplet, as seen here.

Use the GDA software (see later section) to activate the measurement. It is analysing the whole image and determining the widest vertical region. By following the plotted size in the graph (cyan lines usually), you can determine if the drop is expanding or contracting.

If the droplet expands the RH delivered by the HC1 needs to be lowered. If, on the other hand, the droplet shrinks the RH needs to increase and the procedure continues the same way. After any change a couple of minutes are required and before starting with a new droplet. This process should be followed until a RH is found that permits the droplet to be stable over a 5 minute period. This is the RH_i and would be the starting RH for every subsequent experiment.



Please note that given the fact that the HC1 is an open system and around 4 L/min of humid air is being blown over the sample the RH equilibrium solutions with small amounts of salts or precipitants often require 99.9 % RH and still have a tendency to dry. As the intention of the experiment is to dry the crystals and, as the water available in the solvent channels of the samples is heavily hydrogen-bonded, this is not normally a problem and most crystals will be stable. As mentioned earlier, it is best to dehydrate the samples slightly than to hydrate them.

Mounting and preparing samples for dehydration

Mounting samples

Mounting samples is relatively easy but requires a bit of practice. We recommend using MicroMeshes from either Molecular Dimensions or MiTeGen as they prevent samples from swirling uncontrollably inside the standard loops, which makes data collection impossible. It also makes the removal of mother liquor (see later) much easier.

Below are some general pointers to make your experiment easier:

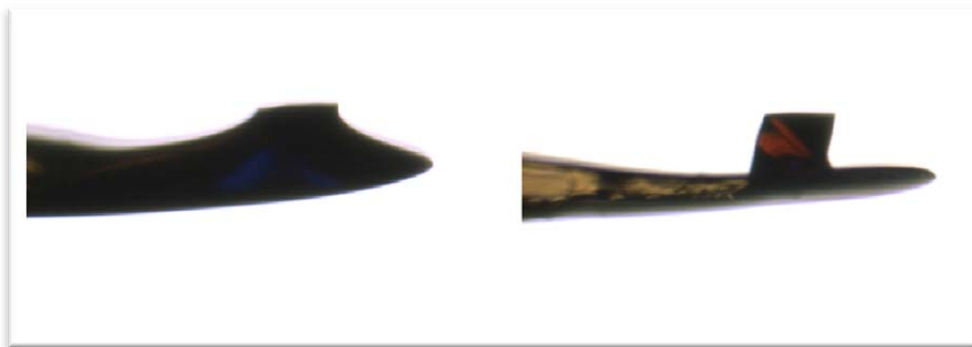
- Larger samples (>100 microns) are useful if crystals are radiation sensitive, as they offer multiple data collection points
- Adjust the size of the mesh to match the size of the crystal
- If more than one sample is mounted try to avoid them overlapping in the beam
- Long needles are easier to collect if they are aligned just off the main goniometer axis.
- Remember the position and the side of the mesh where crystals are mounted
- The same mesh can be reused several times

- Wash meshes thoroughly before mounting new samples to avoid radiation damage caused by trapped free radicals
- Meshes are relatively rigid and sometimes crystals need to be moved with nylon loops prior harvesting with the mesh.
- Pre-centre the mesh prior harvesting to prevent mounting the samples out of the airstream
- Samples are at room temperature so are not rigid and can be dropped if knocked when mounting.
- Don't need to avoid dragging excess mother liquor as this will be removed later and it will shield the samples from dehydration between the drop and the nozzle.
- Try to mount the samples swiftly but gently to prevent dehydration thus without dropping them.

Wicking excess mother liquor

This step is the most alien to crystallographers. It is important as it ensures crystals do not move, results are more consistent and equilibration times are shorter. The objective is to leave the crystal free of mother liquor such that changes in the vapour equilibrium around them can directly impact the crystal. This prevents skins forming around the crystals, movements, cracking during the dehydration process. It may need skipping if crystals are very sensitive to humidity changes (liquid acting as dampener) or if samples prove to be very sensitive to handling. Nevertheless, it is always best to leave as little mother liquor as possible around the sample for better results during dehydration and to aid in future cryo-cooling. The process is as follows:

- Mount the crystal onto the mesh and carefully but swiftly transfer the sample to the goniometer
- Rotate the pin so that the crystal is facing up
- With the crystal in position, remove the excess mother liquor **from below** with a wick



Crystal before and after wicking the excess mother liquor

As the crystal is protected by the mesh the wicking process can be relatively ruthless (as long as the crystals are not very delicate plates or needles) and should be done until the shape of the crystal is clearly visible and void of liquid (see picture above). It is better losing a couple of samples at this stage rather than spending one hour dehydrating mother liquor around a crystal and never seeing its effect on the crystal itself. If the crystals are very small it is sometimes difficult to remove all traces of liquid but, as the crystals are small, they often dehydrate well as they don't hold too much liquid around them. Surface tension should be able to hold most samples to the mesh but this process may require a bit of practice the first time.

Possible wicks include a simple rolled sheet of tissue paper, electron microscopy wicks, wooden xtal wicks or any other material. An easy way of doing this is to roll a tissue paper around a finger and use this to touch the base of the mesh.

Performing diffraction and burn tests

This initial stage is important and designed to determine how long and under what conditions crystals survive room temperature data collection. This is achieved by collecting consecutive identical images and observing the diffraction pattern with each consecutive image. By observing the empirical lifetime of the samples, transmission, exposure time, oscillation angle and oscillation range can be determined. Data collection parameters should be such that indexable data can be obtained but so that the minimum number of images and beam transmission are used. The recommended starting omega angle would be one that is offset to any of the main crystal axis (45 degrees of the plane of the mesh is usually good). Omega oscillation of 1 or 2 degrees is ideal, independent of the detector used, to help with assessing resolution limit, unit cell parameters and crystal mosaicity. Low transmission and time are very important starting at 1% 0.2s and increasing only if necessary for correct indexing.

Once the general parameters are defined, we recommend collecting the same image/s separated by 30-60 seconds of waiting time. This process will help determine how for many runs of images crystals produce a usable diffraction pattern: the crystal lifetime. 5-10 images are typical but it could range from 0-2 to 20-30. This should be repeated a couple of times to get a reliable feel for the sample behaviour.

Key at this stage is to analyse the lattice parameters in comparison to the pre-existing data at cryo-conditions. Do they pack in the same space group? Do they have larger/smaller cell parameters? Are any of them very different to cryo? Is the resolution limit higher/lower than expected? How low is the mosaic spread? Are they good enough for structural solution if you could collect a full dataset at room temperature?

Performing an initial dehydration experiment

Advantages and disadvantages

Because the experiment is online, there is direct feedback of the behaviour of each crystal, as dehydration occurs and data is being collected, prior to cryo-cooling. On the other hand, the amount of beamtime will restrict how many samples can be tested and thus results may not have a high statistical significance. The method relies on your crystals being moderately radiation hard at room temperature (RT) as this determines how many data points can be analysed for each sample. The key to this experiment is to expose the crystals to as little radiation as possible while permitting successful indexing so that progress can be correctly assessed. This should be done by monitoring lattice parameters and relative resolution as a measure of change and not only focus on improvement.

Performing the initial set of dehydration experiments

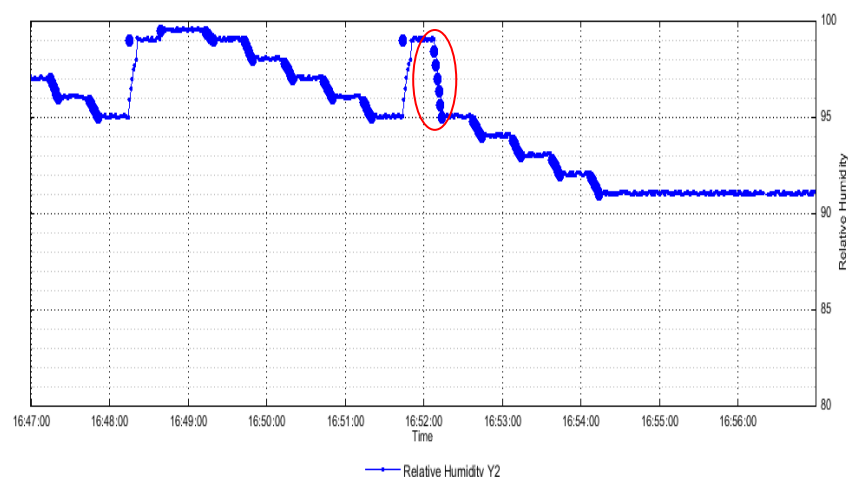
Once RH_i and the lifetime of the crystals have been determined and there is a sensible set of data collection parameters, the dehydration experiment can now begin. This initial stage is solely designed to determine if dehydration causes a change in the diffraction properties of your crystals. The initial stages are more approximate and coarse and later, if any effects are observed, the protocols may require finer adjustment.

Starting at RH_i , using the RH_{target} and considering the lifetime of the crystals a set of dehydration protocols can be tested. We recommend starting at dehydration rates of 1 % per minute, steps of 1 % and allowing the crystals to stabilise for around 4-10 minutes prior data collection. The crucial aspect is to make sure that each image or set of images is analysed and unit cell parameters are monitored in relation with the dehydration state. Furthermore, the mosaicity of the samples is a clear indication of change and the relative diffraction resolution and reflection profiles can be used to assess any changes.

The recommended initial data points for this experiment are:

Target Point	RH Step	Waittime	Slope	Xtal #
$RH_i - 1$	1	4 mins	1 %/min	1
$RH_i - 2$	2	4 mins	1 %/min	1
$RH_i - 3$	3	4 mins	1 %/min	1
$RH_i - 4$	4	4 mins	1 %/min	2
$RH_i - 5$	5	4 mins	1 %/min	3
$RH_i - \dots$		4 mins	1 %/min	6
$RH_i - 15$	5	4 mins	1 %/min	7
$RH_i - 20$	5	4 mins	1 %/min	8

- The experiment will take different directions depending on what is observed
- If time allows do 2 or more crystal repeats per step
- If experiments are successful up to a certain RH further samples can be conditioned directly to this humidity before exposing to radiation and thus extending subsequent experiments
- If a change is observed fine tune the dehydration needed with smaller RH steps as required
- Changes may need longer than 5 minutes to complete so optimise the equilibration time accordingly
- Some effects are reversible and improvement may also be observed with rehydration
- Keep flux down to avoid radiation damage
- Remember to return to RH_i at the end of dehydrating each crystal



Example of the RH trace whilst an experiment was being carried out. During this experiment two crystals were analysed: The first one died after 5 steps at 95% RH and the second one was dehydrated directly to 95 % (red circle) and data was collected from this point until it reached 91%

Following the progress of dehydration

Following the process of dehydration is crucial but can be difficult. Often with low transmission and limited images the automated indexing struggles. Also, as the crystals dehydrate they often move, crack so visual comparison can be deceiving. When crystals undergo transitions they often become highly mosaic, with streaky Bragg reflections and impossible to index. The extent of change can only be assessed once they finish their transition if they reach a more ordered state. Finally, the effect of dehydration is convoluted by the detrimental effect radiation damage may be having on the sample at the same time.

We recommend users concentrate their efforts in firstly observing the images by eye and comparing the previous and the current state. If lattices get smaller this will show in expansion of the spot separation and order and disorder can be seen in the spot profiles. If the images have sufficient reflections for indexing the automated indexing may be able to pick the right lattice but as diffraction worsens with radiation it may be necessary to index manually picking the individual spots and forcing certain lattices despite high penalties.

Performing an offline HC1 experiment

Advantages and disadvantages

Because this experiment is performed offline there is no direct feedback. For this reason it is important to analyse more data points, repeated multiple times in order to get a good idea of what happens to the crystals. On the other hand, there is no data being collected until the samples are cryo-cooled so, despite being able to observe the real improvement achievable not being limited by radiation damage, results may be masked by the changes caused by the cryo-cooling process. This method is essential when the sensitivity of your crystals to radiation is so high that RT data collection is not practical. Finally, as there is no pressure for the time used offline, experiments can be performed for as long as they require thus undertaking a potentially more thorough experiment.

Determining the initial RH_i and a RH_{target}

As was described earlier already there is a need to determine the value of relative humidity that the HC1 needs to deliver in order for your crystals to be stable. This is the RH_i of your mother liquor. Follow previous explanation for reference.

Performing a set of dehydration experiments

Not being able to analyse the data whilst the experiment is running requires harvesting crystals at the different RH of interest. Once each crystal has been dehydrated to the point of interest it needs to be cryo-cooled and then stored for future analysis. We recommend you cool the crystals in perfluoropolyether oil or in a standard cryo that would normally be used with these samples. Beyond a RH of 95-94% samples may not need cryo-protectant⁴ but it may still be worth testing adding oil or cryo-protectant to them.

Suggested data points for this experiment are:

Target Point	RH Step	Waittime	Approx. total time	Slope	Xtal #
$RH_i - 0.1$	0.1	4 mins	5 mins	0.1%/min	1
$RH_i - 0.2$	0.1	4 mins	10 mins	0.1%/min	2
$RH_i - 0.5$	0.1	4 mins	25 mins	0.1%/min	3
$RH_i - 1$	1	4 mins	5 mins	1%/min	4
$RH_i - 2$	1	4 mins	11 mins	1%/min	5
$RH_i - 3$	1	4 mins	17 mins	1%/min	6
$RH_i - 4$	1	4 mins	23 mins	1%/min	7
$RH_i - 5$	1	4 mins	29 mins	1%/min	8
$RH_i - 6$	1	4 mins	35 mins	1%/min	9
$RH_i - 7$	1	4 mins	41 mins	1%/min	10
$RH_i - 8$	1	4 mins	47 mins	1%/min	11
$RH_i - 9$	1	4 mins	53 mins	1%/min	12
$RH_i - 10$	1	4 mins	59 mins	1%/min	13
$RH_i - 15$	5	5 mins	30 mins	2%/min	14
$RH_i - 20$	5	5 mins	40 mins	2%/min	15
$RH_i - 30$	10	5 mins	21 mins	5%/min	16

This would fill one unipuck to later test on the beamline. A good set to cool would be 1-2 pucks with samples frozen with no cryo, 1-2 of samples frozen with cryo and 1-2 of samples frozen with oil. This would require between 32 to 96 samples and between 1 to 6 days.

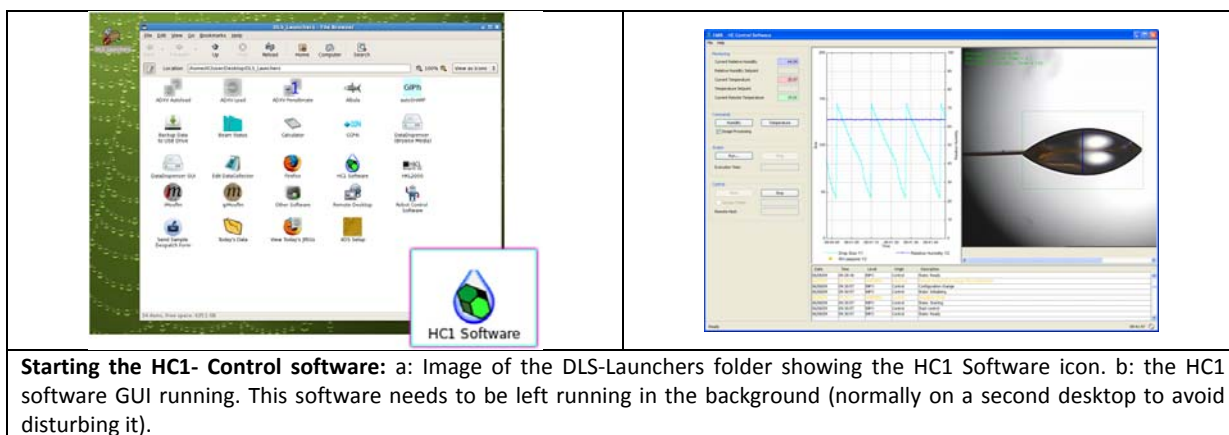
⁴ Pellegrini, E., D. Piano, *et al.* (2011). "Direct cryocooling of naked crystals: are cryoprotection agents always necessary?" *Acta Crystallogr D Biol Crystallogr* **67**(10): 902-906.

SECTION II: USING GDA TO UNDERTAKE A DEHYDRATION EXPERIMENT

Starting the HC1 remote software

For GDA to control the HC1 requires HC1 software to be running in the background. If this software stops running (you close it by mistake or it gets killed) you will not be able to do operate the HC1 until you launch it again and re-start the GDA server. Your local contact will set this up for your visit.

To start the software find the DLS_Launchers folder on your Desktop and double click on the “HC1 Software” icon . To avoid closing we recommend opening it in the secondary linux workstation, different to the one running GDA. If the software is running correctly there will be an image from the on-axis viewing system and a graph displaying temperature and the actual RH of the device (in blue).

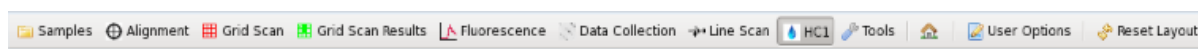


Starting GDA and the HC1 perspective

Once the software is running GDA can be restarted. It is advisable to re-start the server if you are re-establishing communication with the software. Once the GDA client is started your welcome perspective should look like this:

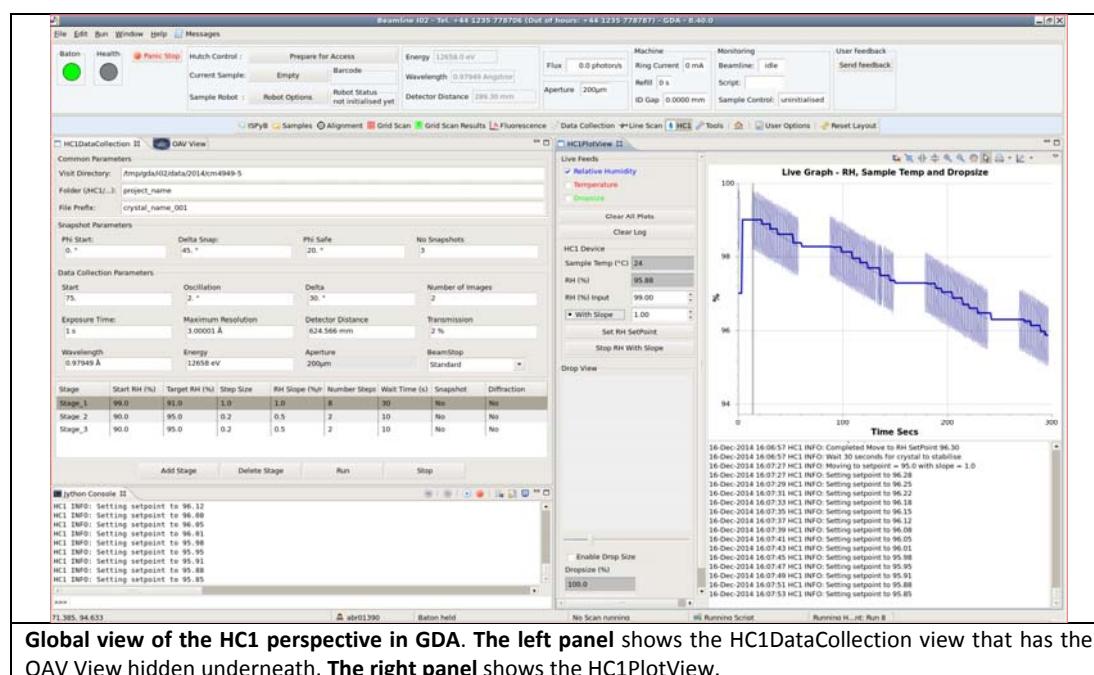


Once the GDA client is open click on the HC1 icon to open the corresponding perspective. When this or any other perspective is active you can migrate between perspectives using the menu bar.



The HC1 perspective has three views. The first one is the standard “OAV View” that give you control of the sample image and centring. The second one is the “HC1PlotView” used to manually alter the sample humidity in either single steps or via smooth gradients, to follow the progress of humidity change before and during an experiment and to aid in determining the initial relative humidity. The last view “HC1DataCollection” is used to input the experimental parameters for the dehydration experiment. This includes the alterations to humidity, the parameters to collect pictures and the x-ray diffraction collection parameters.

Alter the perspective layout such that it looks a bit like this so you can undertake all aspects of dehydration in a coordinated way.



Global view of the HC1 perspective in GDA. The left panel shows the HC1DataCollection view that has the OAV View hidden underneath. The right panel shows the HC1PlotView.

Using the software to change the airstream relative humidity

To manually alter and monitor humidity you can use the controls at the left of the HC1PlotView. The greyed fields are the read-back values for both sample temperature (which you cannot change) and the airstream relative humidity. The requests for relative humidity can be inputted in the “RH (%) Input” field. To move to a new RH immediately use the Set RH SetPoint button. In order to move to a new RH using a gradient you first need to define the slope (measured in % per minute), then toggle the button to change mode (from “Without Slope” to “With Slope”) and then push the Set RH SetPoint button in order to to change to a new humidity with a gradient at the defined speed. If you make a mistake when requesting a single step change simply input the correct value and click the Set RH SetPoint button. If you input the wrong target RH or an incorrect

slope click the Stop RH With Slope (this will stop at the last request) and then correct the error and start again.

HC1PlotView

Live Feeds

☒ Relative Humidity

☐ Temperature

☐ Dropsize

Clear All Plots

Clear Log

HC1 Device

Sample Temp (°C) 24.22

RH (%) 96.87

RH (%) Input 99.00

☐ Without Slope 1.00

Set RH SetPoint

Stop RH With Slope

HC1PlotView

Live Feeds

☒ Relative Humidity

☐ Temperature

☐ Dropsize

Clear All Plots

Clear Log

HC1 Device

Sample Temp (°C) 24.08

RH (%) 96.3

RH (%) Input 99.00

☒ With Slope 1.00

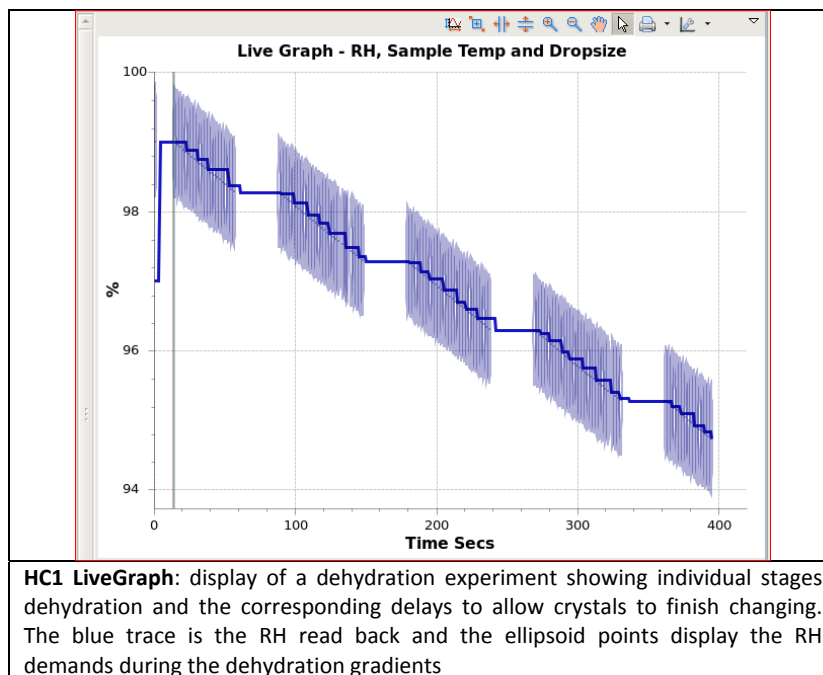
Set RH SetPoint

Stop RH With Slope

Manual controls for the HC1 humidity airstream: Left panel shows the controls ready for a changes from a current point around 97% to a new point of about 99%. As the Without Slope is displayed this will happen in a single fast step. The right panel shows a similar change but as the toggle is on the With Slope mode is active and thus the change will happen at the input slope speed of 1 % per minute.

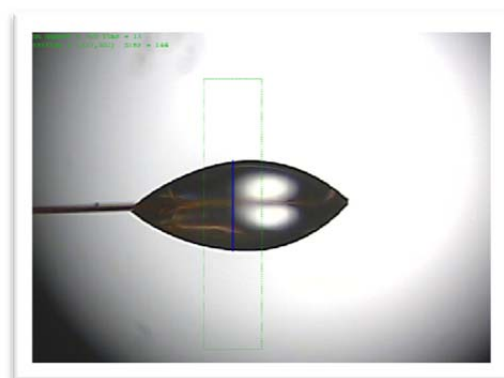
Monitoring Relative Humidity

To follow the status of the device there is a constant read back of the calculated RH displayed on the RH (%) grey window and all requests and actions are displayed in a bespoke log panel. This read back value is also plotted in a Live Graph. This graph will update constantly and can also display sample temperature and drop size. To change the plotted values toggle on and off the required variables. The graph can be autoscaled and rescaled. The display and logs can be cleared for clarity and will resume with the new values or actions.



Using the software to determine the RH_i and the RH_{target}

RH_i or initial relative humidity is the empirical relative humidity at which a crystal is stable in the HC1 airstream. To begin the process set the humidity of the airstream to a value between 90-99%. For a description on how to obtain a good approximate initial value see the previous sections of this document. Once the HC1 reaches the desired relative humidity, by hand mount a 500-800 μm diameter Kapton loop on the goniometer, centre to the cross hair and align the loop such that the loop is perpendicular to the viewing system. Use the controls within the OAV View to do this. Once the loop is in place dispense a small 0.2-1 μl of the solution of interest such that it forms a droplet like the one shown. Once the drop is in place push the reset drop size button (in the HC1PlotView). If this now reads 100% then activate the read the drop size. This will now start monitoring the size of the droplet and display an overlay showing the calculated maximum dimension of the drop. The progress of the drop can be monitored using by activating the **Dropsizes** toggle in the HC1PlotView.



☐ Enable Drop Size Dropsizes (%) 100.0 Reset Drop Size	☒ Enable Drop Size Dropsizes (%) 100.0 Reset Drop Size	Live Feeds ☒ Relative Humidity ☐ Temperature ☐ Dropsizes Clear All Plots Clear Log
HC1 RH_i measurements: dropSize controls showing the disabled/enabled toggle for drop size measurements. Graph controls to use to display or clear the dropsizes measurements.		

Clear or rescale the plot such that you can follow the behaviour of the droplet. If the droplet is expanding or contracting over 1-2 minutes then un-select the Enable Drop Size, change the RH of the airflow, dry the Kapton loop and, once the humidity is stable at the new humidity point repeat the process.

If the drop size measurement is a bit unstable try adjusting the zoom, lighting and droplet angle such that you get a more stable measurement. Lighter backgrounds may work better. For extra details/help with this process ask your local contact.

Setting up and Running a Dehydration Experiment/s

A dehydration experiment is split into **stages** containing identical individual **steps** within them. This allows for simple or complex experiments to be defined. Once the input parameters are defined the user can simply run the whole method and the individual dehydration stages with their individual images will all be undertaken automatically. The user can concentrate on monitoring the progress of the experiment by carefully visual inspection of the diffraction images and crystal snapshots and (if possible) by following the crystallographic cell parameters and mosaicity.

All the parameters needed for the experiment can be input within the HC1DataCollection view. Here you will be able to define the dehydration stages and steps, the common parameters (folders & names), snapshot parameters and data collection parameters.

Common Parameters

Visit Directory: /tmp/gda/i02/data/2014/cm4949-5

Folder (/HC1/...): project_name

File Prefix: crystal_name_001

Snapshot Parameters

Phi Start: 0. ° Delta Snap: 45. ° Phi Safe: 20. ° No Snapshots: 3

Data Collection Parameters

Start: 75. Oscillation: 2. ° Delta: 30. ° Number of Images: 2

Exposure Time: 1 s Maximum Resolution: 3.00001 Å Detector Distance: 624.566 mm Transmission: 2 %

Wavelength: 0.97949 Å Energy: 12658 eV Aperture: 200µm BeamStop: Standard

Stage	Start RH (%)	Target RH (%)	Step Size	RH Slope (%/r	Number Steps	Wait Time (s)	Snapshot	Diffraction
Stage_1	99.0	91.0	1.0	1.0	8	30	No	No
Stage_2	90.0	95.0	0.2	0.5	2	10	No	No
Stage_3	90.0	95.0	0.2	0.5	2	10	No	No

Add Stage Delete Stage Run Stop

The HC1DataCollection view: Different sections within the HC1DataCollection view: **Common Parameters**, aimed at inputting the data architecture. **Snapshot Parameters**, used to define how to record crystal pictures. **Data Collection Parameters**, used to input the X-ray diffraction data collection parameters. The **Method section**, where the desired dehydration stages and steps can be programmed and where the buttons to edit run and stop the methods are.

Dehydration Experimental Parameters

To create a stage press the Add Stage button at the bottom of the HC1DataCollection view. Edit the input stage parameters that you require.

- **StartRH:** The humidity you want to start at.
- **TargetRH:** The humidity you want to end the stage at.
- **Step Size:** The RH increment/decrease you want each individual step in the stage.
- **RH Slope:** The rate in %/minute at which you want to reach each individual step.
- **Number of Steps:** (Normally calculated) the resulting number of steps the stage will have.
- **Wait Time:** Seconds that you will allow the crystal to stabilise before you either take a picture, take diffraction images and/or you move to the next step in the stage.
- **Snapshot:** toggle (YES/NO) to indicate if you would like to take a picture at the end of each step
- **Diffraction:** toggle (YES/NO) to indicate if you would like to collect X-ray diffraction images at the end of each step

Stage	Start RH (%)	Target RH (%)	Step Size	RH Slope (%/r	Number Steps	Wait Time (s)	Snapshot	Diffraction
Stage_1	99.0	91.0	1.0	1.0	8	60	Yes	Yes

Add Stage
Delete Stage
Run
Stop

HC1 Experiment Planning (single stage): Section of the HC1DataCollection view where dehydration methods can be set. In the first case the method would generate a stage going from 99 to 91 % RH. There will be 8 steps (98%, 97%,... 91%). The speed will be 1%/min. After reaching the desired humidity at each step the software will wait 60 seconds, then take pictures and finally take diffraction data.

Stage	Start RH (%)	Target RH (%)	Step Size	RH Slope (%/r	Number Steps	Wait Time (s)	Snapshot	Diffraction
Stage_1	99.0	93.0	6.0	1.0	1	120	Yes	No
Stage_2	93.0	91.0	0.2	0.5	10	60	Yes	Yes

Add Stage
Delete Stage
Run
Stop

HC1 Experiment Planning (multiple stages): Example with two stages (99-93 % RH and 93-91 % RH). The first stage is done in a single step of 6 % jump at 1 %/min. After waiting for 2 minutes then it collects pictures and moves to the next stage. The following stage takes it from this point to 91 % RH at 0.5 %/min in 10 steps of 0.2. This time waiting for 1 minute and collecting both pictures and data for 10 different steps (92.8, 92.6, 92.4, ... 91 % RH).

Common Parameters (folder and image names)

In order to define the image names and the data location you need to edit the common parameters.

- **Visit Directory:** current working directory for your visit (you cannot edit)
- **Folder (/HC1/...):** folder and sub-folders where you would like data to be written to
- **Phi Safe:** oscillation angle at which you would like to keep your crystal during dehydration and/or waiting times.

HC1DataCollection
OAV View

Common Parameters

Visit Directory: /tmp/gda/i02/data/2014/cm4949-5

Folder (/HC1/...): project_name

File Prefix: crystal_name_001

Snapshot Parameters

HC1 Experiment Planning (Common Parameters): In the current example data is written into the /tmp/gda/i02/data/2014/cm4949-5 directory. The particular images are going to be placed in a subfolder /HC1/project_name and each image will be called crystal_name_001.

Snapshot Parameters (they control the picture settings)

- **Phi Start:** angle at which you would like to take your first picture
- **Delta Snap:** oscillation jump you would like to do between your pictures
- **Phi Safe:** oscillation angle at which you would like to keep your crystal during dehydration and/or waiting times.
- **No. Snapshots (max4):** total number of pictures you would like to take.

Snapshot Parameters			
Phi Start: 0. °	Delta Snap: 45. °	Phi Safe 20. °	No Snapshots 3

HC1 Experiment Planning (snapshot parameters): In the current example it would take 3 pictures at 0, 45 and 90 degrees. Between individual events the sample should be kept at 20 degrees to avoid it slipping out of the mesh.

Data Collection Parameters (controls X-ray data settings)

- **Start:** angle at which you would like to collect the first image.
- **Oscillation:** oscillation angle that you would like to collect each X-ray frame.
- **Delta:** oscillation jump you would like to do between consecutive frames.
- **No. of Images:** total number of diffraction frames you would like to take at each dehydration step.
- **Exposure time:** exposure time for each X-ray frame
- **Maximum Resolution:** resolution you would like the edge of the detector to be at (distance/energy dependant)
- **Transmission:** % of beam you would like to use for the X-ray frames.
- **Beamstop:** pull-down the desired beamstop position

Data Collection Parameters			
Start 75.	Oscillation 2. °	Delta 30. °	Number of Images 2
Exposure Time: 1 s	Maximum Resolution 3.00001 Å	Detector Distance 624.566 mm	Transmission 2 %
Wavelength 0.97949 Å	Energy 12658 eV	Aperture 200µm	BeamStop Standard ▼

HC1 Experiment Planning (X-ray diffraction data parameters): At the end of each step the software would collect 2 diffraction images the first one at 75 and the second at 105 degrees. Each 2 degrees wide (75-77 and 105-107) and exposed for 1 second with 2 % beam. As the energy is 12658 keV the detector wold is set to 624.5 mm such that the diffraction images are collected with 3 Å at the edge of the detector. The beamstop would be standard and the aperture would be a 200 µm diameter.

Running and stopping a Dehydration Method

Once all the required parameters are complete you can start the dehydration method by simply pushing the Run button. This should start the method, clear the plots and continue until it is all complete. If you would like to stop at any point just press the Stop button. This many not stop immediately as if any actions are underway it will first complete them and then stop. It will finish taking snapshots, it will finish collecting some diffraction images and then it will exit before starting anything else.

A log detailing only the key method actions is being plotted during a method for you reference. Please use it as a reference to troubleshoot any issues.

Add Stage Delete Stage Run Stop
HC1 Experiment Planning (multiple stages): Example with two stages (99-93 RH and 93-91 RH). The first stage is done in a single step of 6% jump at 1%/min. After waiting for 2 minutes then it collects pictures and moves to the next stage. The following stage takes it from this point to 91% RH at 0.5%/min in 10 steps of 0.2. This time waiting for 1 minute and collecting both pictures and data for 10 different steps (92.8, 92.6, 92.4, ... 91 % RH).